SYMPOSIUM ON AMEBIASIS*

Panel Discussion

THE SEROLOGY OF AMEBIASIS

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DR. GEORGE HEALY. Serology has a useful place in the diagnosis of amebiasis. We all agree that in cases of liver abscess, serology is very good; in cases of invasive intestinal amebiasis, it is also useful.

When we talk about how good something is we might profitably begin by showing its limitations. I call to your attention a seroepidemiologic study on sera from Cherokee Indian school children in North Carolina (Figure 1). These children had prevalence rates of 50% for *Ascaris lumbricoides* infections, 39% for *Trichuris trichiura* infections, and a variety of intestinal protozoa. In 1965 we found 11% of them infected with *Entamoeba histolytica*. The frequency-distribution curve of the titers suggests that there were probably no cases of invasive amebiasis. This supposition is supported by the low positive serology, a fact corroborated by the experience of the physicians in the Indian Hospital. The point to be noted is that serology and stool positivity do not necessarily have to agree; they may mean different things. Figure 2 demonstrates the converse of this; two populations were examined by stool examinations and serology, with invasive amebiasis confirmed in population B by a high incidence of serologic as well as stool positives. The seroepidemiologic curve extended farther out and had a peak titer of 1:1024, which is consistent with our results in present or recently acquired clinical amebiasis.

As the use of serology in amebiasis becomes more accepted and commercial diagnostic preparations become available to the individual physician within the next year or two, some caution has to be taken in the interpretation of serologic results. In some of the tests used in

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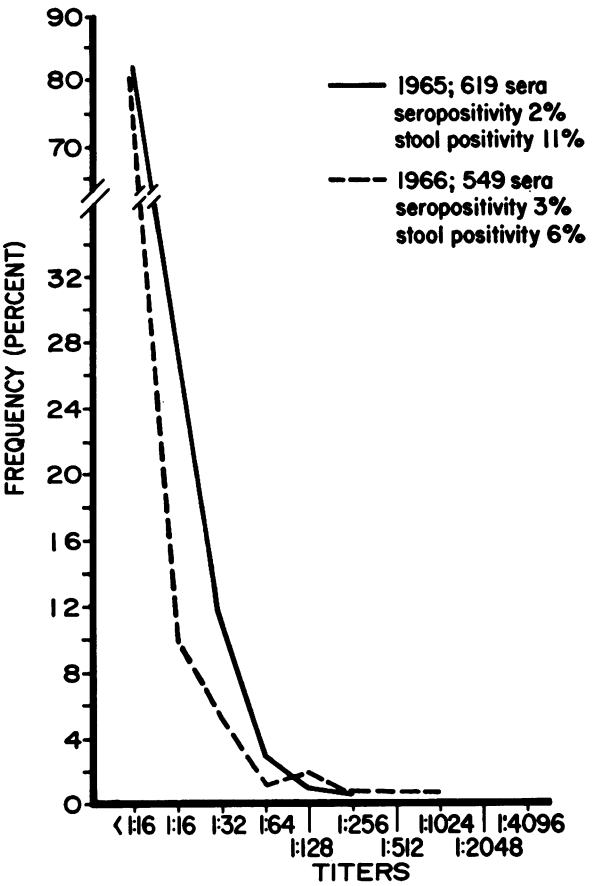

Fig. 1. Frequency distribution of IHA titers to *Entamoeba histolytica* antigen. Cherokee Indian school children. Reproduced by permission from Healy, G. R., Kagan, I. G. and Gleason, N. N. *Health Lab. Sci. 7*:109-16, 1970.

amebiasis serology, the titers drop slowly over a long period of time after chemotherapy. The titer reported on the serum from an individual may be due to an antibody response which was stimulated by experience with the organisms weeks or months prior to the test. The current symptoms in the patient may not be at all related to the serology of amebiasis.

DR. ELSDON-DEW. We have been studying amebiasis in Durban for a long time. Initially we tried doing complement-fixation tests but our results were just a bit confusing. We obtained very good results in

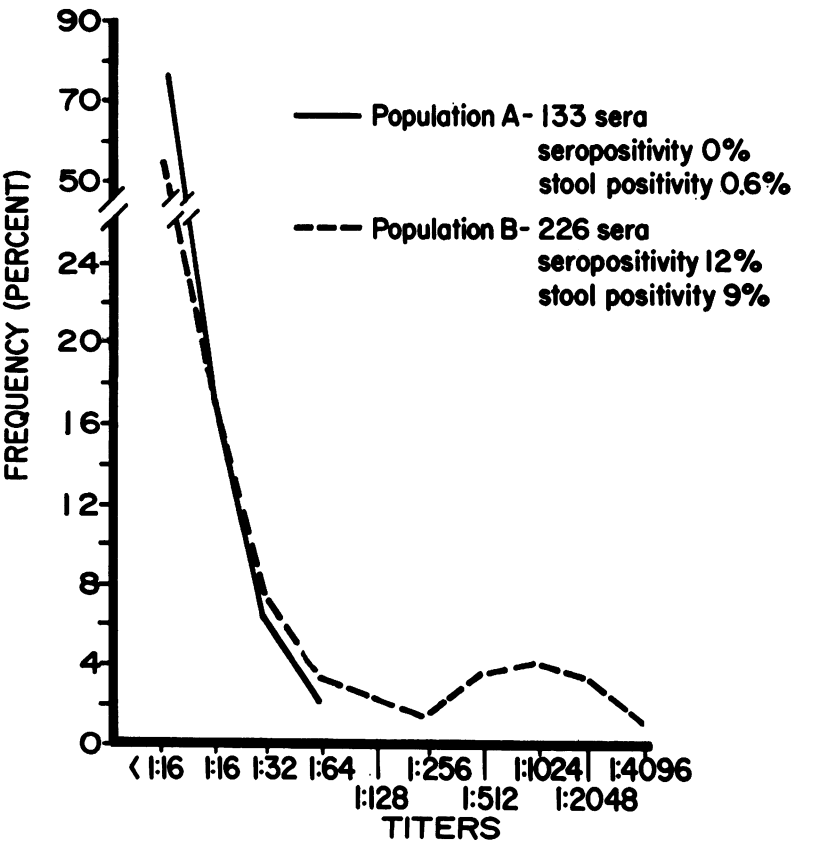

Fig. 2. Frequency distribution of IHA titers to *Entamoeba histolytica* antigen. Calion, Ark. Reproduced by permission from Healy, G. R., Kagan, I. G. and Gleason, N. N. *Health Lab. Sci.* 7:109-16, 1970.

cases of liver abscess (I think there was approximately a 98% positivity of the sera from liver abscesses). We had cases, however, in which we could not find the ameba after careful search and in which there were positive reactions. Sera from cases of amebic dysentery gave us, I think, a bit more than 80% positivity or thereabout. This led us to believe that though there was something useful in serology (we did not know what it was), it was well that we should attempt to do some developmental studies. Complement fixation and indirect hemagglutination (the latter at that time was very much in its youth) were good! Fluorescent antibody tests were not available at that time. I determined,

however, that we should use the simplest possible system that would allow us to analyze our results critically. To that end, happily, just at that time the Ouchterlony agar-gel procedure came into use.

This is a very good technique since by means of it one can identify individual antigen-antibody reactions. It was about this time that we started working with Dr. S. John Powell and his clinical group. This association gave us an inestimable advantage in that we could obtain adequately documented cases with which to evaluate our tests. This was fortunate, for a test must be evaluated against some sort of known standard, and we knew the exact clinical history of every case; our number of these cases now exceeds 30,000. To make a long story short, we found that the gel-diffusion test is very good. We interpret our findings in this way: a positive reaction implies present or past invasion with *E. histolytica*. We find that many cyst passers show no detectable antibodies at all. In these cases we assume the amebas have remained in the commensal phase and have not traversed the barrier of the mucosal surface. One may be leading with one's chin in this statement, but I am pretty sure this is correct.

Last year I was able to carry out another study in a mental institution in Holland. We had all the parasitological findings, and we found that a large proportion of cyst passers were serologically negative. When we checked our findings with the clinical histories (here we had patients who were captive and we were able to obtain their complete medical histories) the serology fitted entirely.

So it may be asked: "What is the place of serology in the diagnosis of amebiasis"? I think that in acute amebic dysentery, the microscope is still the tool. This method is fast and it is neat! But there are times when serology is, practically speaking, a little faster, as in liver abscesses. Our physicians in Durban are pretty good at clinical diagnosis but one may come up against a situation in which it is not known whether a palpable liver is in fact due to amebas or to something else. Here a positive serology test will indicate that it *might* be due to amebas. A negative serology test will give one a 98% certainty that it is not due to the ameba. We must remember that the probability of a person having residual antibodies from previous infection depends also on his environment. Consequently one has to have, or should have, as absolutely essential information, some concept of the prevalence of antibodies in individual populations. Bear in mind that this information, the seroepi-

demiology of amebiasis, is probably the most accurate method we may have of estimating the importance of invasion by amebas in any area. It tells us nothing of the number of people who harbor amebas; it does tell us (or give us a concept) of the number of people whose tissues have been invaded by amebas, and I think this is much more valuable information than stool findings alone.

DR. KERRISON JUNIPER: My laboratory is now in the process of analyzing the results of studies of more than 5,000 serum samples from about 4,000 patients from hospitals in the Little Rock area. We have used the indirect hemagglutination (IHA) test as our main means of screening patients serologically for amebiasis, then performing complement-fixation (CF) tests and agar-gel (GEL) diffusion tests on positively reacting serum samples.*

The IHA test was considered clinically significant (positive) at titers of 1:128 or above. One patient with extraintestinal amebiasis had a positive IHA reaction at a titer of 1:262,144 but most of the titers of patients with amebiasis ranged from 1:128 to 1:8,192, with no significant difference between extraintestinal and invasive intestinal cases.

The CF test was considered clinically significant at titers of 1:16 or above. One patient with extraintestinal amebiasis had a CF test positive at a titer of 1:1,024, but most patients with amebiasis had titers of 1:16 to 1:256, with no significant difference between extraintestinal and invasive intestinal disease.

The agar-gel double-diffusion test was performed according to Crowle's microslide method on 10 microliter samples. Most patients reacting in this test showed at least two precipitin bands; one showed as many as eight when first seen.

Of 16 patients with extraintestinal amebiasis, 88% gave positive IHA, CF, and GEL tests. This somewhat low incidence of positive reactors (most reports list 95 to 98% positive) was caused by two patients with classical clinical findings of amebic abscess of the liver, but in whom all the serological tests were negative. The incidence of positive tests in intestinal amebiasis for the IHA, CF, and GEL tests respectively were as follows: 63 invasive cases—98, 85, and 86%; 28 noninvasive symptomatic cases—61, 56, and 54%; and 32 asymptomatic cases—58, 58, and 52%

*Parke-Davis and Company supplied axenically grown *Entamoeba histolytica* antigen for these studies.

Although the IHA test was the most sensitive one, more unexplained positive reactions were encountered with it and the test often remained positive for periods as long as one to two years after cure of the disease. The CF and agar-diffusion tests correlated better with the presence of clinically active amebiasis, and both tests tended to revert to a normal reaction within about six months after cure, with a few exceptions.

I have found these three serological tests to be exceedingly valuable in the clinical diagnosis of amebiasis. Because of its sensitivity, the IHA test can be used as a screening method for clinically significant amebic infection. A positive reactor should be considered a suspect, and appropriate intensive stool examinations should be obtained. It is important to realize that a negative serological test for amebiasis does not exclude active amebic disease; and that serological testing does not replace adequate examination of the stool. The CF and GEL tests are useful in assessing the clinical significance of a positive IHA reaction. These serological tests do not replace skilled clinical judgement, and patients should not be given antiamebic treatment simply because of a positive test.

DR. HEALY. Dr. Powell, would you like to say something of your broad clinical experience and its relation to serology?

DR. POWELL. I agree entirely with what has been said so far about the use of serology in amebiasis. The first point I should like to make, not just from the viewpoint of serology but for the diagnosis of amebiasis, is that the key to diagnosis is awareness. If you do not think of amebiasis as a clinician, you are not going to think about a serologic test. One must be aware of the possibility of amebiasis in a patient. The next point is that you must look at the patient from a clinical point of view. If the patient presents as a typical case of invasive amebiasis you are unlikely to need the serological test. Our present laboratory methods and other precedures for diagnosis are perfectly adequate. For example, I deplore waiting for serological results in order to be absolutely certain that you are dealing with a liver abscess which is clinically obvious and in need of urgent aspiration.

Nevertheless serology can be exceedingly helpful. I have no hesitation in saying that in the particular hospital in which I work we have so much amebiasis that the serological test for amebiasis is the most valuable and the most reliable serological test that we do for any disease in our area. There is no doubt that the serological methods now avail-

able for the diagnosis of amebiasis are both highly sensitive and highly specific for detection of past and present invasive disease.

I want to say a word from the clinician's viewpoint about the danger of titer levels. Clinicians are very apt to think that because one person has a much higher titer than somebody else that therefore he has more amebiasis. This is incorrect. The level of the titer is not necessarily an indication of the degree of tissue invasion, and it seems to us from our studies that people vary quite considerably. Some are high "responders," some are moderate "responders," some are low "responders," and just a few seem to be negative "responders" as far as their titers are concerned.

There is no doubt that antibodies may persist for a good many years after cure. Therefore the presence of a positive test on the serum of a patient does not necessarily indicate active infection, nor after therapy does it mean that treatment has failed. There is generally a slow fall in the titer level after successful treatment. The length of time for the fall depends, I think, on the initial height of the titer before treatment. If you start off with a very high titer, it takes longer, in general, for this to fall or become negative than if you start off with a low titer. Of course, what we need and do not yet have is a test which will indicate active infection as opposed to past or cured infection. Such a test would make life much simpler.

The last point I should like to make is that we do have a great need for a simple, quick test which can be done in all those regions of the world where sophisticated laboratory methods are not available. And I can assure you from my experience around the world that many of our present tests are not likely to be used very widely in areas of invasive amebiasis. The facilities just aren't there. We need, and we hope we are close to having, a simple test that clinicians can do and understand .

COMMENT FROM THE FLOOR.. In our hands the IHA test was not too useful in Malaysia because we found the positivity rate in some areas to be 30 to 40%. The other thing that wasn't emphasized was the use of the IHA test as an epidemiological tool. Through the years, people have done stool surveys and no one really has shown what these surveys mean in terms of invasive amebiasis. Yet here we seem to have a very quick, easy, odorless method for discovering whether an area is having invasive amebiasis or noninvasive amebiasis.

DR. ELSDON-DEW. We have done some fairly extensive seroepidemiological studies, not only on populations but on conditions. Let me first deal with the populations and then I shall give you some idea as to the kind of results we obtained locally in Durban and over a wider range in South Africa. In the paper I have already presented I refer to the apparent difference (I said apparent) in amebiasis in the white and black populations. This is reflected markedly in the serological results. A random hospital population of Bantu had approximately 16% positive. The Durban Bantu volunteer blood donors, a slightly higher social stratum than the general hospital patient, had 9% positive. The whites in Durban are less than 1% positive. Black donors in Johannesburg are between 1 and 2% positive. Blood donors in Cape Town, less than 1%. This is illustrative of the kind of information you get.

On a slightly different scale, I thought that it would be of value to study the serology of people of different origins under uniform living conditions. I had the opportunity to do this because the gold-mining companies in the Transvaal operate blood-transfusion services and they have many donors of varying origins. I therefore tested the specimens of blood from these people. I was able to classify them as to whether they were Xhosa, Zulu, Sotho, and all the other native tribes, in addition to categorizing them by their geographic origins. The people from Botswana (formerly Bechuanaland), showed an unexpectedly high prevalence of antibodies. Botswana is in a remote portion of the country. I made inquiries, and it appeared that amebiasis was indeed quite common there. Thus, as far as I was concerned, serology had uncovered an area where I did not know that amebiasis existed. This was illustrative of the use of seroepidemiology.

There are other aspects of seroepidemiology. In one of our early studies we were able to study patients who had ulcerative colitis. We did not have any patients of our own thus afflicted and so we had to test some from other areas. These proved completely, utterly negative. We had in the same group in the same series, many cases of Dr. Powell's, those of postamebic dysentery or postamebic colitis; they were 100% positive. So, seroepidemiology can be applied not only to people and places, but also to disease states.

QUESTION. I have two questions to put to you. Persons at the National Institutes of Health (NIH) who have been doing indirect

hemagglutination tests for us in supected liver abscess have said that a negative reaction may indicate very early development of an abscess, and one may have to repeat the test, perhaps a few weeks later. I should like to know what relations these possible false negatives have in the diagnosis of very early acute liver abscesses? What is the present state of development and the potential use of soluble antigen fluorescent antibody test (SAFA) in amebiasis? Could it be applied in a large scale screening by use of the fluorimeter?

DR. HEALY. Let me answer the second question myself first. There is a movement today in serology, as there has been in clinical chemistry, toward the development of automated procedures. The soluble antigen fluorescent antibody test is being evaluated by a number of laboratories. My laboratory is collaborating with Dr. Roy Taylor of Fort Sam Houston, Texas, in comparisons of the IHA and SAFA tests in amebiasis. I think unfamiliarity with the machine is one of the basic problems.

I think the concept of having automated procedures by which one can test a great many specimens and get faster, more reproducible results is at the very forefront of development in serology. Dr. Kenneth Walls of our Parisitology Section at the Center for Disease Control has successfully adapted one of the automated devices used in clinical chemistry (the Technicon Autoanalyzer) for complement fixation tests for some of the parasitic diseases. The extent to which the SAFA test will be used as a screening device as well as for diagnosis depends upon its widespread use and evaluation as to its sensitivity and specificity. I refer your first question about false negatives to Dr. Powell.

DR. POWELL. I think it all depends, really, how early your patients with the liver abscess are presented to you. By and large our African patients in Durban tend to present fairly late. In the case of gel diffusion-precipitin studies, if one studies early, initial sera on admission one finds about 98% positive. If one repeats the study 10 or 20 days later, then one may increase that positivity rate by about 1%.

DR. JUNIPER. Our number of cases is small, but we have had one patient with a liver abscess, with symptoms of only two weeks' duration who had a positive serologic test; another with symptoms of four weeks duration had a positive test. I do not recall any of our invasive-colon patients with a negative serologic test who had a positive one later. All of our patients have been positive or negative serologically

throughout the course of the illness. However, it is interesting that very frequently we do see a significant rise in titer two or three weeks after the initial specimen, particularly after the patient has been started on treatment. In a few instances when patients, for various reasons, have not been treated, we have seen a continued rise in titer over a period of a number of months.

DR. ELSDON-DEW. I am going to theorize a little. You have all seen pictures of amebas in hepatic tissue. I do not like to use the word "liver abscess" because the lesion is not truly a liver abscess; it is an area of necrosis. One of the main pathologic features is a lack of tissue response; this rather suggests that the human host does not recognize the ameba as foreign. Yet we obtain a positive serologic response. The only way I can explain this is by assuming that some of the amebas die and release antigenic material from within. I do not think the human body recognizes the outside of the ameba as foreign. Hence it may well appear that we have to wait for the ameba to die for an antigenic stimulus to result. I think amebas die rather quickly, some of them even in the intestine.

DR. HEALY. As a parasitologist I find it interesting that amebas can "colonize" in the liver and produce, in some cases, a very large tissue abnormality, the abscess, and yet not initiate antigenic stimulation sufficient to produce an antibody response. It may be that they do colonize, grow, and multiply, and do not die and release any antigen until the abscess is fairly well developed, but I do not know how fast the abscess grows.

DR. JUNIPER. The thing that worries me about our two serologically negative cases is whether we might be dealing with another cause of "aseptic" necrosis of the liver. I must admit that the patients responded very nicely to emetine treatment. This would seem to indicate that the disease was amebic, but it is conceivable that there might be another etiology. It astounds me that a negative serologic reaction can occur in the presence of an amebic abscess.

DR. POWELL. We have found *E. histolytica* in the aspirate in one or two serologically negative cases. But this is certainly very uncommon. As to the question of how long it takes to develop a liver abscess, I do not know the whole answer, but we have seen liver abscesses develop in babies five or six weeks old.

QUESTION. Dr. Powell: on the question of titer. If, as you point

out, the higher titer is not necessarily a manifestation of greater tissue invasion, what should we take as a significant titer?

DR. POWELL. I think you have to work that out on the basis of the control in your local population. Dr. Healy showed some nice biphasic curves, and I think he found that for the local populations which these represented, a significant titer was 1:128.

DR. HEALY. I do not think one need be concerned about a diagnostic titer in too many instances. It has been my experience in the diagnostic laboratory that sera from cases of amebiasis, particularly liver abscesses, generally have fairly high IHA titers, much beyond the minimum level of 1:128, we established, as Dr. Powell indicated. Our minimum diagnostic titer is 1:128 based on seroepidemiologic evidence and clinical information in our original studies. We recently tested a serum, by IHA, from a young man in Florida who had a liver abscess. The titer of his serum, tested several times, fluctuated between 1:128 and 1:256. The physician aspirated a liter of pus from his liver. He responded very well to emetine. Such a low titer is unusual; the majority of sera from cases of severe clinical amebiasis are positive at titers of 1:1024-2048 and many 1:32,000 or greater.

QUESTION. Dr. Healy, would you suggest that two or more specimens be collected so that a rising titer might be detected?

DR. HEALY. No. As has been pointed out, it has been the experience of several workers that differences in titer are not apparent on all acute-convalescent status such as exists in virology or with some direct agglutination tests that are used in bacteriology. The same titer or a two-fold dilution difference may persist from a few weeks to several months or more and then drop slowly.

QUESTION. Will you discuss intradermal reaction in amebiasis?

DR. ELSDON-DEW. The intradermal reaction is testing something entirely different from what we have been discussing. It is not a test of circulating antibodies. We know that there is something happening but I do not think we really have adequate information yet. Frankly, when we have such easy tests as the serologic ones, I should say that we should use serologic rather than skin tests for now. Undoubtedly we shall learn more about skin-test reactions in due time and then be better able to answer that question properly. Dr. Powell raised a point a moment ago and I should like to comment on it here if I may.

We have studied the gel-diffusion test, in depth, to the extent of

isolating the various antigenic fractions concerned. The gel-diffusion test suffers from one clinical disadvantage (it is not an epidemiologic disadvantage); that is, it takes a little time to complete gel diffusion. You have to wait for antibodies to diffuse toward the antigens and, in fact, one cannot report an unequivocal negative reaction in less than 48 hours. This is a clinical disadvantage. Another disadvantage is that a certain amount of technical expertise is necessary. The test is not something which can be done ad lib or "in the bush," so to speak.

In Durban we have been trying for a long time to find some simple method of serologic testing. We have developed a latex test which on comparison with the gel diffusion gave an agreement within 1%, which is as good, I think, as one may expect to get between two serologic tests. We are not absolutely sold on the present latex test as it is, and we are still awaiting reports on whether the test is really as good in other people's hands as it has proved in ours.

QUESTION. Dr. Elsdon-Dew, were your population studies done with the gel-diffusion test?

DR. ELSDON-DEW. Yes, all with gel diffusion.

DR. HEALY. I think that the serologic diagnosis of amebiasis is "coming of age," so to speak. The World Health Organization has lately become interested in evaluating the effectiveness of amebiasis serology on a global basis, and we have recently completed some studies in five countries. There are companies interested in marketing diagnostic kits, and commercial amebiasis antigens or test kits have shown great promise in preliminary work. These kits will be available, I am sure, because there has developed a keen interest in them and they are needed. At the present time I know of at least three companies who are testing commercial amebiasis antigens.

QUESTION. Has anyone done much work on children in terms of their titers? We found, and you confirmed this, Dr. Healy, that some of the children did not have a high rate of negativity in terms of titers as might be expected if one considers that they are primary responders rather than secondary responders.

DR. HEALY. I have not had any extensive experience with young children.

DR. POWELL. One of our pediatricians in Durban has been doing a study using gel diffusion and, in the case of liver abscesses, the percentage of positives in children in the same, or approximately the same, as

in adults. In the case of amebic dysentery there is a far greater percentage of negatives in very young children. Children tend to approximate the percentage of positives in adults after four years of age. Certainly, in very young children, the percentage of positives is less.

DR. JUNIPER. From a slightly different aspect: whenever we have an individual with invasive disease, we try to obtain serum from all members of the family. Sometimes there are as many as 10 children in these families, and the incidence of positive reactors is very high. In some instances the sera from the entire family will react, whereas we may demonstrate *E. histolytica* in the stools of only one half the family members. However, I am impressed that children become seropositive to amebic invasion very readily.

QUESTION. I should like to ask about the standardization and origin of the antigens in view of the fact that in early isolates host red-cell antigen would be present. Is there an attempt to standardize antigens and perhaps indicate how long an ameba should be cultured before the antigen would be considered standardized?

DR. HEALY. Most of the ameba cultures used for growing organisms for antigens are quite old, and I do not believe there is any host antigen associated with them. For example, in our laboratory we are growing, in Diamond's axenic medium, the HK-9 strain of *E. histolytica* isolated in 1952. This strain was brought to this country by Dr. W. W. Frye in what I have been told was a very smelly flight because he had several culture tubes strapped to his body when he brought back the several K strains from Korea in 1952. I do not think many workers are growing amebas or using amebas for antigens directly isolated from a patient, either from stools or from liver abscess. Years ago investigators made antigen by alcoholic extract of amebas in liver abscess fluid or organisms from severe amebic dysentery, but today most people use antigens from amebas that are grown in culture without any host antigens.

QUESTION. Would you comment on the use of serology in amebic hepatitis.

DR. POWELL. I think it is an important one. One of the most valuable contributions that serology could make for us is in helping to delineate precisely what are the conditions of invasive amebiasis. I think that there are two most fertile fields for this. One is in delineating amebic hepatitis, if it exists as a condition, and the other is in helping us to

sort out the vague condition of chronic, sympotomatic, internal, intestinal amebiasis which is another very variable diagnosis.

DR. VICTOR G. HEISER. It is customary at symposia such as these to call upon an old Methuselah who by personal experience can connect the distant past with the present. My experience with amebic dysentery began in 1902 in Egypt, then in India, and then, on a very large scale, in the Philippines.

We soon found that at the time the United States government had taken over the administration of the Philippines, large numbers of Americans were employed by the Philippine government. It is safe to say that nearly half these employees and their families developed dysentery, although not always amebic dysentery. Then we found that they had exactly the same experience in other countries. Other tropical countries showed the same incidence, the British in India, Malaya, and Borneo, the French in Indochina, the Dutch in Java, the Australians in New Guinea.

Another clinical characteristic developed. In most of these countries which were dominated by either Americans or Europeans, it was customary on the approach of the hot season, to move the government to higher altitudes. This was almost invariably accompanied by large outbreaks of epidemics of diarrhea; here again not necessarily all outbreaks were due to amebic dysentery. It is well to mention that in the early years, perhaps up until 1908, it was not recognized that there were pathogenic and nonpathogenic amebas. We recognize that today, of course. In the Philippines, standard treatment for amebiasis included a huge enema, frequently given daily, of quinine solution. This treatment took two to three weeks to cure a routine case. The other alternative was ipecac, so you can imagine how unpopular the treatment for amebic dysentery was. Then Sir Leonard Rogers came along with his reports of the success of emetine in treating amebic dysentery.

It may be of interest to mention that at the close of World War I it was anticipated that a large number of amebic cases would come back with the returning troops. In anticipation of this possibility, the Rockefeller Foundation organized a unit that was intended to deal with this problem should it occur. Fortunately the returning troops had very little dysentery and there was no need to use the facilities which the Rockefeller Foundation was prepared to make available.